WEST Search History

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DATE: Thursday, March 18, 2004

Hide?	Set Name	Query	Hit Count
		DB=USPT; PLUR=YES; OP=AND	
☐	L1	6024961.pn.	1
☐	L2	L1 and aro$2	1
☐	L3	L1 and pho5$	0
☐	L4	L1 and pho$5	1
☐	L5	L4 and insert$	1
☐	L6	L5 and plasmid$	1
☐	L7	L1 and safe$	1
☐	L8	6383496.pn. and safe$	1

END OF SEARCH HISTORY

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L4: Entry 2 of 4 File: USPT Mar 30, 1993

DOCUMENT-IDENTIFIER: US 5198464 A
**** See image for Certificate of Correction ****
TITLE: Method and compositions for helmintic, arthropod ectoparasitic and acaridal infections with novel agents

CLAIMS:

17. An animal feed premix composition for the prevention, treatment or control of helminthic, arthropod ectoparasitic or acaridal infections in meat-producing animals, said animal feed premix composition comprising: an edible carrier; and a prophylactically, therapeutically or pharmaceutically-effective amount of the fermentation broth or whole mash of microorganism Streptomyces cyaneogriseus noncyanogenus, having deposit accession number NRRL 15773, or a mutant thereof, containing antibiotic agents produced by the microorganism or the mutant thereof; or or the pharmaceutically and pharmacologically acceptable salts thereof; wherein said agents have characteristic spectra as shown in FIGS. I-LVII of the attached drawings.

WEST Search History

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DATE: Thursday, March 18, 2004

updated search 3/18/04

Hide?	Set Name	Query	Hit Count
		DB=USPT; PLUR=YES; OP=AND	
☐	L1	(5451519 or 4798791 or 5595736 or 6383496).pn.	4
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=AND	
☐	L2	dam.clm. or (adenin$ near2 methylase).clm.	3703
☐	L3	L2 and (alter$ or mutant$ or mutation$ or mutagene$ or modifi$ or attenuat$ or enhanc$).clm.	386
☐	L4	L3 and (excipient$ or carrier$ or diluent$ or buffer$ or solution or capsule or pharmaceutic$).clm.	53

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 4 of 4 returned.

1. 6383496. 18 May 99; 07 May 02. Recombinant vaccines comprising immunogenic attenuated bacteria having RPOS positive phenotype. Curtiss, III; Roy, et al. 424/200.1; 424/258.1 424/93.2 435/252.3 435/252.8 435/471 435/897. A61K039/02 A61K048/00 C12N015/74 C12N001/21.

2. 5595736. 27 Mar 95; 21 Jan 97. Compounds and methods for treatment of thromboembolic disorders. Berg; David T., et al. 424/94.64; 424/94.63 435/212 435/226. A61K038/46 A61K038/49 C12N009/48 C12N009/64.

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Terms	Documents
(5451519 or 4798791 or 5595736 or 6383496).pn.	4

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Search Results - Record(s) 1 through 50 of 53 returned.

☐ 1. 20040009939. 03 Mar 03. 15 Jan 04. Methods of enhancing immune induction involving MDA-7. Chada, Sunil, et al. 514/44; 424/93.2 514/12 A61K048/00 A61K038/17.

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Terms	Documents
L3 and (excipient$ or carrier$ or diluent$ or buffer$ or solution or capsule or pharmaceutic$).clm.	53

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L1: Entry 1 of 4 File: USPT May 7, 2002

US-PAT-NO: 6383496
DOCUMENT-IDENTIFIER: US 6383496 B1

TITLE: Recombinant vaccines comprising immunogenic attenuated bacteria having RPOS positive phenotype

DATE-ISSUED: May 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Curtiss, III; Roy	St. Louis	MO		
Nickerson; Cheryl A.	River Ridge	LA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Washington University	St. Louis	MO			02

APPL-NO: 09/ 314062 [PALM]
DATE FILED: May 18, 1999

PARENT-CASE:
This is a continuation-in-part of application Ser. No. 08/970,789, filed Nov. 14, 1997, now U.S. Pat. No. 6,024,961.

INT-CL: [07] A61 K 39/02, A61 K 48/00, C12 N 15/74, C12 N 1/21

US-CL-ISSUED: 424/200.1; 424/93.2, 424/258.1, 435/471, 435/252.3, 435/252.8, 435/897
US-CL-CURRENT: 424/200.1; 424/258.1, 424/93.2, 435/252.3, 435/252.8, 435/471, 435/897

FIELD-OF-SEARCH: 424/200.1, 424/93.2, 424/258.1, 435/252.3, 435/252.8, 435/471, 435/879

PRIOR-ART-DISCLOSED:

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Search Selected Search ALL Clear

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ART-UNIT: 1648

PRIMARY-EXAMINER: Mosher; Mary E.

ATTY-AGENT-FIRM: Thompson Coburn LLP

ABSTRACT:

Attenuated immunogenic bacteria having an RpoS.sup.+ phenotype, in particular, Salmonella enterica serotype Typhi having an RpoS.sup.+ phenotype and methods therefor are disclosed. The Salmonella have in addition to an RpoS.sup.+ phenotype, an inactivating mutation in one or more genes which render the microbe attenuated, and a recombinant gene capable of expressing a desired protein. The Salmonella are attenuated and have high immunogenicity so that they can be used in vaccines and as delivery vehicles for genes and gene products. Also disclosed are methods for preparing the vaccine delivery vehicles.

31 Claims, 16 Drawing figures

First Hit

L4: Entry 1 of 53 File: PGPB Jan 15, 2004

PGPUB-DOCUMENT-NUMBER: 20040009939
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040009939 A1

TITLE: Methods of enhancing immune induction involving MDA-7

PUBLICATION-DATE: January 15, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Chada, Sunil	Missouri City	TX	US	
Pataer, Abujiang	Houston	TX	US	
Mhashilkar, Abner	Houston	TX	US	
Ramesh, Rajagopal	Sugarland	TX	US	
Roth, Jack	Houston	TX	US	
Swisher, Steve	Fresno	TX	US	

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	COUNTRY	TYPE CODE
Board of Regent, The University of Texas System				02
Introgen Therapeutics, Inc.				02

APPL-NO: 10/ 378590 [PALM]
DATE FILED: March 3, 2003

RELATED-US-APPL-DATA:
Application is a non-provisional-of-provisional application 60/404932, filed August 21, 2002,
Application is a non-provisional-of-provisional application 60/370335, filed April 5, 2002,
Application is a non-provisional-of-provisional application 60/361755, filed March 5, 2002,

INT-CL: [07] A61 K 48/00, A61 K 38/17

US-CL-PUBLISHED: 514/44; 514/12, 424/93.2
US-CL-CURRENT: 514/44; 424/93.2, 514/12

REPRESENTATIVE-FIGURES: NONE

ABSTRACT:

The present invention relates to compositions and methods for the enhancing or inducing an immune response against an immunogenic molecule by indirectly activating PKR. More specifically, immunotherapy is improved by co-administering a MDA-7 polypeptide with an immunogenic molecule against which an immune response is

desired. Such immunotherapies include cancer vaccines, and compositions thereof are described.

[0001] This application claims the priority of U.S. Provisional Patent Application Serial No. 60/404,932, filed Aug. 21, 2002, U.S. Provisional Patent Application Serial No. 60/370,335, filed Apr. 5, 2002, and U.S. Provisional Patent Application Serial No. 60/361,755 filed Mar. 5, 2002, the entire disclosures of which are specifically incorporated herein by reference.

Hit List

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Search Results - Record(s) 1 through 1 of 1 returned.

☐ 1. Document ID: US 6024961 A

L6: Entry 1 of 1 File: USPT Feb 15, 2000

DOCUMENT-IDENTIFIER: US 6024961 A
TITLE: Recombinant avirulent immunogenic S typhi having rpos positive phenotype

Brief Summary Text (7):
Work directed toward achieving avirulence in microbes for use in vaccines has utilized attenuating mutations in biosynthetic genes, regulatory genes and/or genes involved in virulence. (See Doggett and Brown, supra). One such regulatory gene which has been mutated as a means for achieving avirulence has been the rpoS gene. The rpoS gene encodes an alternative sigma factor, RpoS, which is known to regulate the stationary phase expression of over 30 genes (for review, see Loewen and Hengge-Aronis, Annu Rev Microbiol 48:53-80, 1994). The rpoS gene has been shown to contribute to the virulence of Salmonella enterica serotype Typhimurium (also referred to as Salmonella typhimurium) in mice by RpoS regulation of chromosomal as well as plasmid-borne genes (Fang et al., Proc Natl Acad Sci 89:11978-11982, 1992; Norel et al., FEBS Microbiol Lett 99:271-276, 1992; Kowarz et al., J Bacteriol 176:6852-6860, 1994). Similarly, RpoS is thought to contribute to the virulence of Salmonella typhi in humans by an action on chromosomal gene determinants of virulence, inasmuch as these microbes do not possess the virulence plasmid present in S. typhimurium (Robbe-Saule et al., FEMS Microbiol Let 126:171-176, 1995; Coynault et al. Mol Microbiol 22:149-160, 1996). Mutant rpoS S. typhimurium strains have been shown to be avirulent (Fang et al, supra) and capable of eliciting protective immunity in mice (Nickerson and Curtiss, Abstracts of the 96th General Meeting of the American Society for Microbiology B-141:179, 1996; Coynault et al., Mol Microbiol 22:149-160, 1996). As a result, it has been suggested that rpoS mutants may be attractive candidates for the development of vaccines (Nickerson and Curtiss, supra).

Drawing Description Text (7):
FIG. 6 illustrates the construction of plasmid vectors and bacterial strains with the defined .DELTA.phoPQ23 mutation.

Drawing Description Text (8):
FIG. 7 illustrates the construction of plasmid vectors and bacterial strains with the defined .DELTA.asdA16 mutation.

Drawing Description Text (9):
FIG. 8 illustrates the pYA3433 plasmid.

Detailed Description Text (3):
The rpoS gene product contributes to the virulence of Salmonella typhimurium in mice, at least in part, by regulating expression of chromosomal gene determinants

of virulence and is believed to contribute to S. typhi virulence in humans through a a similar mechanism. Much of the work that has led to the development of live S. typhi vaccines for immunization of humans has relied upon studies using strains of S. typhimurium tested in mice. These S. typhimurium strains cause an invasive infection in susceptible mice that resembles typhoid in humans. (Carter and Collins, Collins, J. Exp. Med. 139:1189-1203; Hohmann et al., Infect Immun 22:763-770, 1978; Coynaut et al. Molecular Microbiol. 22:149-160, 1996). Furthermore, the role of the rpoS gene in the invasiveness and virulence of Salmonella typhimurium is relevant to to the invasiveness and virulence of Salmonella typhi which lack a virulence plasmid inasmuch as strains of Salmonella typhimurium cured of the virulence plasmid have been shown to colonize Peyers patches with efficiency similar to that of the wild-type microorganisms (Gulig and Curtiss, Infect Immun 55:2891-2901, 1987; Hackett et al., J Infect Dis 153:1119-1125, 1986). The results of studies in Salmonella typhimurium, which are thus also applicable to Salmonella typhi, show that the rpoS gene product controls the expression of chromosomally encoded genes which are important for invasiveness and virulence. (Nickerson and Curtiss, Infect and Immun 65:1814-1823, 1997; Kowarz et al, J Bacteriol 176:6852-6860, 1994).

Detailed Description Text (5):
In addition, virulence plasmid-cured derivatives of the rpoS mutant were recovered in lower numbers from murine Peyer's patches than were plasmid-cured derivatives of the isogenic wild-type S. typhimurium. This indicates that RpoS regulation of chromosomally-encoded genes is important for colonization of the murine gut associated lymphoid tissue (GALT) by S. typhimurium.

Detailed Description Text (11):
It is also possible to introduce into Salmonella containing a functional rpoS.sup.+ gene another functional recombinant rpoS.sup.+ gene on a plasmid replicon or integrated into the chromosome to further enhance the expression of genes regulated by the RpoS protein. This might be desirable in certain situations such as, for example, in microbes having diminished rpoS gene expression, i.e., microbes which display nonoptimal colonization of the GALT, or even in microbes where the rpoS gene expression is not diminished but a greater than normal expression is desired.

Detailed Description Text (14):
The rpoS.sup.+ Salmonella strains of the present invention are avirulent by virtue of their containing an attenuating mutation in one or more genes that renders the microorganism avirulent. In a preferred embodiment, the strains have at least two mutations each of which act to attenuate the microorganism and which, in combination, significantly increase the probability that the microorganism will not revert to wild-type virulence. Mutations can be insertions, partial or complete deletions or the like so long as expression of the gene is diminished and virulence is decreased. Attenuating mutations can be in biosynthetic genes, regulatory genes and/or genes involved in virulence. (See Doggett and Brown, supra). Examples of mutations include, but are not limited to a mutation in a pab gene, a pur gene, an aro gene, asd, a dap gene, nadA, pncB, galE, pmi, fur, rpsL, ompR, htrA, hemA, cdt, cya, crp, phoP, phoQ, rfc, poxR, galU and combinations thereof. The skilled artisan will readily appreciate that any suitable gene mutation can be used in the present invention so long as the mutation of that gene renders the microorganism avirulent.

Detailed Description Text (16):
Recently, new methods have become available for producing specific deletions in genes. These methods involve initially selecting a gene in which the deletion is to be generated. In one approach the gene can be selected from a genomic library obtained commercially or constructed using methods well known in the art (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., 1989, Cold Spring Harbor Press, Cold Spring Harbor, N.Y.). Clones containing the gene are isolated from the genomic library by complementation of a strain which contains a mutation in the same gene. Alternatively, when the DNA sequence of the gene is known, selected

primers for the polymerase chain reaction method (PCR) can amplify the gene, often with some flanking sequence, from a sample of bacteria or from purified genomic DNA and the PCR product can be inserted into a cloning vector.

Detailed Description Text (33):
The term gene as used herein in its broadest sense represents any biological unit of heredity. It is not, however, necessary that the recombinant gene be a complete gene as is present in the parent organism and capable of producing or regulating the production of a macromolecule such as for example, a functioning polypeptide. The recombinant gene may, thus, encode all or part of an antigenic product. Furthermore, the recombinant gene can also include DNA sequences that serve as promoters, enhancers or terminators and DNA sequences that encode repressors or activators that regulate expression of a recombinant gene encoding all or part of an antigen. A recombinant gene can also refer to gene fusions which encode polypeptide fusion products. The encoded gene product can, thus, be one that was not found in that exact form in the parent organism. For example, a functional gene coding for a polypeptide antigen comprising 100 amino acid residues can be transferred in part into a carrier microbe so that a peptide comprising only 75, or even 10, amino acid residues is produced by the cellular mechanisms of the host cell. However, if this gene product can serve as an antigen to cause formation of antibodies against a similar antigen present in the parent organism or as a T-cell epitope recognized by T-helper cells, the gene is considered to be within the scope of the term gene as defined in the present invention. Alternatively, if the amino acid sequence of a particular antigen or fragment thereof is known, it is possible to chemically synthesize the DNA fragment or analog thereof by means of automated gene synthesizers or the like and introduce said DNA sequence into the appropriate expression vector. This might be desirable in order to use codons that are preferred codons for high level expression in Salmonella. At the other end of the spectrum is a long section of DNA coding for several gene products, one or all of which can be antigenic. For example, such a long section of DNA could encode 5 to 15 proteins necessary for the synthesis of fimbrial antigens (fimbriae), which mediate adhesion of pathogens to host cells (Baumler et al., supra). The induction of an immune response against fimbriae can provide protection against the pathogen. Thus, a gene as defined and claimed herein is any unit of heredity capable of producing an antigen. The gene can be of chromosomal, plasmid, or viral origin. It is to be understood that the term gene as used herein further includes DNA molecules lacking introns such as, for example, is the case for cDNA molecules, so long as the DNA sequence encodes the desired gene product.

Detailed Description Text (40):
Any of a number of commonly used recombinant DNA techniques can be used in producing the avirulent microbes of the present invention which are capable of expressing a recombinant gene. Following ligation to a plasmid, phage or cosmid vector the recombinant molecules so formed can be transferred into a host cell by various means such as conjugation, or transformation (uptake of naked DNA from the external environment, which can be artificially induced by the presence of various chemical agents, such as calcium ions), including electroporation. Other methods such as transduction are also suitable, wherein the recombinant DNA is packaged within a phage such as transducing phage or cosmid vectors. Once the recombinant DNA is in the carrier cell, it may continue to exist as a separate autonomous replicon or it may insert into the host cell chromosome and be reproduced along with with the chromosome during cell division.

Detailed Description Text (50):
The bacterial strains used in the present studies were constructed using the following general materials and methods. Listings of phages, plasmids and micro-organisms used in constructing the strains are given in Tables 1 and 2.

Detailed Description Text (52):
Bacteriophage P22HTint was used for transduction using standard methods (Davis et

al., A Manual for Genetic Engineering--Advanced Bacterial Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1979). An overnight culture of a donor strain was diluted 1:20 into prewarmed Luria broth, grown for one hour with shaking at 37.degree. C., and then infected with P22HTint at a multiplicity of infection (MOI) of 0.01. The infection mixture was shaken overnight or for approximately fifteen hours. A few drops of chloroform were added to ensure complete complete bacterial cell lysis, and the mixture was allowed to shake an additional ten minutes at 37.degree. C., then centrifuged at 7,000 rpm in a Sorvall SS-34 rotor rotor for ten minutes to remove bacterial debris. The supernatant fluid was extracted and removed to a clean tube with a drop or two of fresh chloroform and stored at 4.degree. C. This method generally provides a phage lysate containing about 10.sup.10 PFU/ml titered on .chi.3000. Tetracycline was used in plates at 12.5 .mu.g/ml to select for Tn10 transductants, Tn10-induced mutations, or merodiploid strains expressing the Tn10-derived tetracycline-resistance genes from a a chromosomally integrated suicide vector. The Tn10 transposon excises from the chromosome at a low frequency, often deleting a portion of the genome flanking the transposon. Cells which undergo an excision event also become sensitive to tetracycline, and can be identified by plating on media containing fusaric acid, which kills tetracycline-resistant bacteria (Maloy and Nunn, J. Bacteriol. 145:1110-145:1110-1112, 1981). Tetracycline-sensitive strains which have lost an integrated suicide plasmid along with the plasmid linked tetracycline-resistance genes can also also be selected on fusaric acid media.

Detailed Description Text (54):
Suicide vectors containing an ampicillin-resistance gene, a sucrose-utilization cassette, and an incP mobilization site were constructed. Mutant genes which have been introduced into these plasmids can be introduced into the bacterial chromosome after transformation, or preferably by conjugation, to generate ampicillin-resistant merodiploids. Such merodiploids can be grown on media containing 5% sucrose to select for the loss of the integrated plasmid along with the ampicillin-resistance and sucrose-utilization genes. Ampicillin-sensitive strains can be phenotypically characterized for the presence of appropriate defined deletion mutant alleles.

Detailed Description Text (58):
.chi.3339 is a wild-type, virulent, animal-passaged isolate of S. typhimurium strain SL1344 described in Gulig et al. (Infect Immun 55:2891-2901, 1987). SF1005 is an rpoS::RR10 mutant derived from S. typhimurium strain ATCC 14028s and containing an ampicillin resistance gene linked to the rpoS::RR10 mutant allele (Fang et al., Proc. Nat'l. Acad. Sci., USA 89:11978-11982, 1992). The mutant rpoS::RR10 allele was moved into .chi.3339 using a P22HTint transducing phage lysate prepared on SF1005 and selecting for ampicillin resistance (Ap.sup.r) due to the presence of the .beta.-lactamase gene (bla) linked to the RR10 insertion in the rpoS gene. The allelic exchange between SF1005 and .chi.3339 was confirmed by Southern blot analysis, and the resulting .chi.3339 rpoS::RR10 mutant derivative was designated as .chi.4973. Transductants were screened for sensitivity to P22HTint by cross streaking with P22H5, a clear plaque mutant. Pseudolysogenic colonies were distinguished from non-lysogens on Evans blue and uranine (EBU) indicator agar (Sternberg et al., Meth. Enzymol. 204:2-43, 1991). Media were supplemented with 50 .mu.g ampicillin per ml when required to select for .chi.4973.

Detailed Description Text (77):
To determine whether the rpoS gene product regulates expression of chromosomally-encoded genes whose products are important for S. typhimurium colonization of Peyer's patches, the wild-type .chi.3339 and rpoS mutant .chi.4973 strains were cured of their virulence plasmids to generate plasmid-cured isogenic derivatives .chi.3340 and .chi.8125, respectively. The ability of these derivative strains to colonize Peyer's patches was examined following peroral administration of .chi.3340 and .chi.8125 in a 1:1 ratio and the data are shown in Table 7 below.

Detailed Description Text (78):
As shown in Table 7, .chi.8125, the virulence plasmid-cured derivative of the rpoS mutant strain .chi.4973, exhibited a reduced ability (ca. 5.1 fold) to colonize Peyer's patches at 5 days postinfection as compared to the colonizing ability of .chi.3340, the virulence plasmid-cured derivative of the wild-type .chi.3339 strain. These data indicate that RpoS regulates expression of chromosomally-encoded gene(s) whose products are important for successful colonization of murine Peyer's patches after oral inoculation.

Detailed Description Text (89):
The generation of chromosomal deletions using transposon Tn10 has been previously described in a wide variety of bacteria, including Salmonella (Kleckner et al., J. Mol. Biol. 116:125-159, 1977; EPO Pub. No. 315,682; U.S. Pat. No. 5,387,744; which are incorporated by reference). Recently, new methods have become available for introducing specific mutations into genes. The gene to be mutated can be selected from a population of clones contained in a genomic DNA library constructed in a cloning vector, or by cloning the amplified product containing all or a portion of the gene into a plasmid using PCR methodology. Mutations introduced into such genes or portions of genes are known as defined deletions and these are constructed using one of two general methods.

Detailed Description Text (91):
Another method employs the use of divergent PCR primers synthesized based upon known DNA sequence either within the gene to be deleted or within DNA flanking the gene. The primers are mixed with a vector containing a cloned gene and subjected to an inverse PCR reaction, resulting in the amplification of the entire plasmid but deleting all or a portion of the target gene (Innis et al., infra). The PCR reaction amplifies upstream and downstream regions flanking a specified segment of DNA to be deleted from the cloned gene and generates a product consisting of the cloning vector and upstream and downstream flanking sequences. The inverse PCR method is preferred because it allows the placement of mutations of any size at any position within a gene of known DNA sequence, and allows the introduction of novel restriction sites to be engineered into the PCR primers or target DNA which then can be used for the subsequent insertion of other cloned sequences. An alternative PCR method for generating defined deletions relies on amplified PCR products which represent portions of the gene or flanking DNA sequence. These are ligated together in a cloning vector to construct the defined deletion mutation.

Detailed Description Text (93):
For example, genomic DNA libraries from wild-type Salmonella typhimurium UK-1 (.chi.3761) can be constructed in a suitable cloning vector such as pNEB-193 (New England Biolabs), which is a pUC19 derivative that carries single sites for the unique 8-base cutters: AscI, PacI and PmeI. Generally, genomic DNA is isolated according to standard methods (Sambrook et al., Molecular Cloning/A Laboratory Manual Second Edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1989). Sau3A1 partially digested genomic DNA is sized on an agarose gel and extracted using commercially available methods in kit form obtained from Promega, Quiagen, or Bio101. DNA fragments between 2 and 6 kb are isolated and ligated into a plasmid first digested with BamHI or BglII, then dephosphorylated using alkaline phosphatase according to the manufacturers' instructions. The resulting plasmid library is then introduced into an appropriate E. coli strain in order to amplify the genomic library and to obtain a population of recombinant plasmids containing random genomic DNA inserts ranging in size from 2 to 6 kb. Relevant clones are isolated from a genomic library by complementation of mutant E. coli or S. typhimurium strains.

Detailed Description Text (94):
Where the DNA sequence of a gene is already known, PCR primers are synthesized and the gene and often some flanking sequence is amplified using PCR methodology

directly from a sample of bacteria or from purified genomic DNA, and the product, cloned into a plasmid vector such as pNEB-193. Thus, where the gene sequence is known, screening a genomic library is not required.

Detailed Description Text (96):
The pir-dependent R6K replicon has been used by numerous investigators and is one of the most reliable suicide vectors available for allele replacement. Replication of the R6K plasmid requires the pir gene product. A pir-dependent plasmid will not replicate in a pir.sup.- host bacterium, and so the presence of a defined deletion mutation on a pir-dependent plasmid will allow for the selection of rare events in which the plasmid has integrated into the host chromosome within a homologous region region flanking the deletion constructed on the plasmid. This event will confer some selectable phenotype upon the strain into which the plasmid has integrated, because even though the plasmid cannot replicate, the integration event provides a mechanism of stable maintenance of the elements on the plasmid. Antibiotic-resistance elements are generally used to select for the presence of the integrated plasmid, and can be selected from genes which encode resistance to ampicillin, kanamycin, chloramphenicol, gentamicin, spectinomycin and tetracycline, and others well known in the art. The host strain which contains a defined deletion along with an integrated suicide vector is characterized as a merodiploid, since it contains two different alleles of the same gene. Generally, the deletion constructed on the vector will represent a gene deletion and the integrated product on the chromosome will have the structure characterized by the presence of a wild-type allele flanking one end of the integrated vector, and the defined deletion mutant allele at the other end of the vector. Other constructions are well known in the art.

Detailed Description Text (98):
Following the introduction of any defined deletion mutant allele into a strain, phenotypes associated with the mutant gene are characterized using standardized tests well known in the art. These tests include determination of phenotypic reversion frequency, confirmation of deletion by Southern blot or PCR, agglutination by O-group specific antisera, production of complete LPS, presence of flagellar H antigen, motility, plasmid content and confirmation of auxotrophies.

Detailed Description Text (99):
Mutant strains may be shown by Southern blot to possess a loss of genetic material corresponding to the region deleted, as revealed by a mobility shift of DNA relative to the wild-type and the defined deletion mutant allele constructed on a plasmid. PCR analysis of mutant strains significantly reduces the time required for confirming the presence of defined deletions since no DNA isolation is required and results can be completed in less than one day. The PCR method also allows the identification of erroneous recombination events or retention of delivery vector sequences, revealed as mobility shifts or the production of multiple DNA fragments other than those expected upon gel analysis of PCR products.

Detailed Description Text (100):
After construction, strains with defined deletion mutations are fully evaluated for properties associated with the mutation and/or which are important for a strain to be immunogenic as well as avirulent. For example, production of full-length LPS similar to the parental wild-type strain is evaluated using silver stained gels. The confirmation of correct O-antigen is determined by antisera agglutination of mutant cells. Mutant strains are evaluated for positive agglutination using diluted poly H antiserum (Difco) and subjected to motility tests in soft agar motility tubes relative to the parent strains and non-flagellated control strains, .chi.3420 and .chi.3422. Standard clinical API test strips are used following isolation of each mutant strain to obtain fermentation and biochemical data for comparison to parental strains. Growth rates and plasmid content of the mutant strains are also compared to that of parental strains. With S. typhi strains, the plasmid content is not evaluated because the large virulence plasmid present in S. typhimurium is absent in S. typhi (Gulig et al., Infect. Immun. 56:3262-3271, 1987).

Detailed Description Text (101):
Construction of Defined Deletions in phoP, phoQ, and phoPQ genes

Detailed Description Text (102):
The Salmonella phoPQ operon consists of phoP and the adjacent downstream phoQ genes. genes. Defined deletions in the phoP and phoo genes can be constructed using an inverse PCR strategy since the entire nucleotide sequence of the operon and some flanking sequence is known. The DNA sequence reveals the presence and position of restriction sites which can be useful in constructing defined deletions in these genes. The genes can be isolated on a single 2,110 base pair PCR product and cloned into a plasmid vector. The recombinant vector containing the phoPQ gene cassette can can be digested with restriction enzymes to delete most of the phoP gene, leaving the phoQ gene intact. The defined phoP deletion on the phoPQ gene cassette can be inserted into a suicide vector, and introduced into the chromosome of a wild-type phoPQ Salmonella to produce an antibiotic-resistant merodiploid, which can be grown on appropriate media to select for the loss of the integrated plasmid along with the antibiotic-resistance marker. Antibiotic-sensitive strains can be phenotypically characterized for the presence of an appropriate defined deletion phoP mutant allele by screening for the loss of acid phosphatase activity using the agar overlay method of Kier et al. (J. Bacteriol. 130:399-410, 1997). A mutation in either phoP or phoQ is sufficient to confer a PhoP.sup.- phenotype.

Detailed Description Text (103):
Defined deletion mutants in phoQ or in both phoP and phoQ can be generated using a similar strategy, using restriction enzymes to delete defined segments of DNA from either phoQ or from both phoP and phoQ, and introduced into the chromosome on a suicide vector to generate merodiploids, which can be counter selected on appropriate media for the loss of the integrated plasmid and antibiotic-resistance marker, and phenotypically screened for the presence of the relevant defined deletion mutant allele using PCR to verify the genotype.

Detailed Description Text (106):
A defined deletion constructed using either method can be excised from the cloning vector using restriction enzymes and introduced into a suicide vector containing an antibiotic-resistance marker. The resulting recombinant suicide vector containing the defined deletion cya allele can be introduced into the chromosome of a wild-type cya Salmonella strain to generate an antibiotic-resistant merodiploid. The merodiploid would be grown on appropriate media to select for the loss of the integrated plasmid along with the antibiotic-resistance marker. Antibiotic-sensitive sensitive strains would be phenotypically characterized for the presence of an appropriate defined deletion .DELTA.cya-27 mutant allele. MGM-232 and .chi.8217 are two S. typhimurium UK-1 strains with defined .DELTA.cya-27 mutations that were constructed by these methods (see Table 1).

Detailed Description Text (108):
Defined deletions in the Salmonella crp gene can be constructed using a strategy similar to that used for construction of a defined deletion in cya. A recombinant vector can be selected which confers a maltose positive phenotype to an E. coli crp mutant strain when grown on MacConkey maltose media. Divergent PCR primers can be used to delete the known Salmonella crp gene and flanking sequences, and the resulting defined deletion introduced into the chromosome of a wild-type crp Salmonella on a suicide vector to generate an antibiotic-resistant merodiploid. The merodiploid could be grown on appropriate media to select for the loss of the antibiotic resistance and the integrated plasmid, and antibiotic-sensitive strains could be phenotypically characterized for the presence of an appropriate defined deletion crp mutant allele.

Detailed Description Text (112):
Mutations in other genes have also been shown to confer avirulence in Salmonella,

including cdt and pmi alleles. Defined deletions in the pmi gene can be constructed using an inverse PCR strategy, or restriction enzymes. A recombinant vector which confers a mannose-positive phenotype to an E. coli or Salmonella mutant pmi strain when grown on MacConkey mannose media can be used to construct a defined deletion mutant pmi allele using either restriction enzymes or inverse PCR. The defined deletion pmi allele can be inserted into a suicide vector and integrated into the chromosome of a pmi.sup.+ Salmonella to generate an antibiotic-resistant merodiploid, which can be grown on appropriate media to select for the loss of the integrated plasmid along with the antibiotic-resistance gene. Antibiotic-sensitive strains would be phenotypically characterized for the presence of an appropriate defined deletion pmi mutant allele by screening for a reversible rough-smooth phenotype, detecting a smooth phenotype due to the synthesis of LPS O-antigen repeats when grown in the presence of mannose, and a rough phenotype when grown in the absence of mannose detected by the absence of agglutination in the presence of LPS O-antisera.

Detailed Description Text (113):
A defined deletion which confers a Cdt.sup.- phenotype upon Salmonella can be constructed using restriction enzymes to delete DNA associated with this phenotype from a recombinant vector which complements a Salmonella cdt mutant. The mutant allele constructed using this strategy can be inserted into a suicide vector and introduced into the chromosome of a cdt.sup.+ Salmonella to generate antibiotic-resistant merodiploids. Merodiploids can be grown on appropriate media to select for the loss of the integrated vector along with the antibiotic-resistance marker, and antibiotic-sensitive strains can be phenotypically characterized for the presence of an appropriate defined deletion cdt mutant allele.

Detailed Description Text (116):
The construction of the defined .DELTA.phoPQ23 .DELTA.asdA16 S. typhi strains in both the ISP1820 and Ty2 backgrounds involved the use of two suicide plasmids, pMEG-213 containing the .DELTA.phoPQ23 region and pMEG-006 containing the .DELTA.asdA16 region.

Detailed Description Text (117):
The .DELTA.phoPQ23 deletion was obtained by digesting pMEG-068 with EcoRV and TthIIII1 removing the 1103 bp EcoRV-TthIII1 fragment encoding the C terminal end of PhoP and the His region of PhoQ, responsible for phosphorylation of PhoP (FIG. 6). The linearized plasmid was then treated with T4 DNA polymerase and religated to produce pMEG-210 (FIG. 6). The BamHI-XbaI fragment of pMEG-210 containing the .DELTA.phoPQ23 deletion was then inserted in the pir-dependent suicide vector pMEG-149 to produce pMEG-213 (FIG. 6). Since pMEG-213 is a mobilizable suicide vector encoding for the selectable marker for ampicillin resistance and the counter-selectable marker, levanosucrase, resulting in sensitivity to sucrose, the plasmid can be conjugated into any strain desired selecting for ampicillin resistance followed by counter-selection for the replacement of the wild-type phoPQ genes with the mutant phoPQ23 in the presence of sucrose. The host strain responsible for the delivery of pMEG-213 was obtained by transforming pMEG-213 into the Pir.sup.+ Asd.sup.- delivery host MGN-617 to produce MGN-758.

Detailed Description Text (120):
Introduction of the defined .DELTA.AasdA16 deletion into any strain requires plasmid DNA to be electroporated into the strain desired followed by selection for tetracycline resistance. The tetracycline-resistant isolates obtained can then be plated on fusaric acid containing media to select for loss of the tetracycline-resistant elements of the suicide vector (Maloy et al., J. Bacteriol. 145:1110-1112, 1981) followed by screening for the Asd.sup.- DAP-requiring phenotype. Both MGN-1038 and MGN-1018 were electroporated with pMEG-006 and tetracycline-resistant isolates obtained. These isolates were then plated on fusaric acid plates containing 50 .mu.g DAP/ml and the isolated colonies obtained screened for the loss of the tetracycline-resistance element of the suicide vector and replacement of the

wild-type asd gene with the .DELTA.asdA16 mutation. Isolates were then confirmed for for tetracycline sensitivity and requirement of DAP. An S. typhi .DELTA.phoPQ23 .DELTA.asdA16 derivative of each strain was selected for further work, which are designated herein as MGN-1191 (ISP1820) and MGN-1256 (Ty2) (See Table 1).

Detailed Description Text (157):
The wild-type rpoS gene can be introduced into the chromosome of MGN-1256 by allelic exchange using the suicide properties of the R6K-based plasmid pMEG-149. Plasmid pMEG-149 is a mobilizable suicide vector which carries a .lambda.pir-dependent R6K replicon and thus requires a host with the pir gene present in trans to allow replication. In addition, pMEG-149 encodes the selectable marker for Ap.sup.r and the counterselectable marker, levanosucrase. Since pMEG-149 derivatives cannot replicate in strains lacking the pir gene, selection of Ap.sup.r transconjugants demands the integration of the plasmid into the chromosome, an event which usually takes place through homology in the inserted fragment.

Detailed Description Text (158):
Plasmid pSK::rpoS contains the entire 1.47 kb S. typhimurium rpoS gene cloned into the EcoRV site of pBluescript/SK. The EcoRI and HindIII fragment containing the wild-type rpoS allele from pSK::rpoS was treated with T4 DNA polymerase and cloned into the SmaI site of the suicide vector pMEG-149 (see FIG. 8). The resulting recombinant vector carrying the wild-type rpoS allele designated as pYA3433, would be introduced into the .lambda.Pir.sup.+ Asd.sup.- delivery host strain, MGN-617. This strain allows the conjugal transfer of any plasmid containing an IncP mob region to any Asd.sup.+ recipient, followed by elimination of the donor on any media lacking diaminopimelic acid (DAP).

Detailed Description Text (159):
Plasmid pYA3433 carrying the wild-type rpoS allele is introduced into the .DELTA.phoPQ .DELTA.asd S. typhi Ty2 strain, MGN-1256, by electroporation. Transformants are selected by spreading on L-agar plates supplemented with DAP (100 .mu.g/ml) and ampicillin (50 .mu.g/ml), followed by incubation overnight at 37.degree. C. Ampicillin-resistant isolates obtained from this transformation procedure will represent the integration of the entire plasmid including the wild-type rpoS allele into the chromosome. Such isolates contain two copies of the rpoS gene, ie. a wild-type and a mutated rpoS allele. The isolates can then be screened on Luria agar supplemented with DAP (100 .mu.g/ml) DAP and containing 5% sucrose to select for loss of the suicide vector sequences. Sucrose-resistant isolates can be screened for sensitivity to ampicillin, for the presence of a functional rpoS allele (using the catalase and/or glycogen synthesis test), for complete LPS and Vi antigen. The rpoS allelic exchange can be further confirmed by Southern blot analysis.

Detailed Description Text (163):
The rpoS.sup.+ vaccine strains are prepared based upon S. typhi strains containing a functional rpoS gene such as ISP1820 using defined deletions as described above in examples 2 and 3 or based upon attenuated rpoS mutant strains such as Ty2 which have a recombinant rpoS gene as described in example 8 above. In the construction of vaccines expressing foreign antigens, the preferred approach is to use a balanced, lethal host-vector system which confers stable maintenance and high-level expression of cloned genes on recombinant plasmids. For this, a chromosomal mutation of the asd gene encoding aspartate .beta.-semialdehyde dehydrogenase is introduced into the RpoS.sup.+ strain to impose an obligate requirement for diaminopimelic acid (DAP) which is an essential constituent of the rigid layer of the bacterial cell wall and which is not synthesized in humans. The chromosomal .DELTA.asd mutation is then complemented by a plasmid cloning vector possessing the wild-type asd.sup.+ gene as well as a recombinant gene encoding the desired foreign antigen. Loss of the plasmid results in DAP-less death and cell lysis. Such balanced-lethal host-vector combinations are stable for several weeks

in the immunized animal host and elicit immune responses against the cloned gene product as well as against Salmonella.

Detailed Description Text (164):
The construction of a defined deletion in the chromosomal asd gene is described in example 3 above. The ISP1820 derivative, MGN-1191 and the Ty2 derivative, MGN-1256, which have .DELTA.phoPQ23 and .DELTA.asdA16 mutations were thus produced. The asd-complementing plasmid containing a recombinant gene encoding the desired foreign antigen can be constructed as described in U.S. Pat. No. 5,672,345. For example, one such plasmid expressing the Hepatitis B virus antigenic nucleocapsid pre-S1 pre-pre-S2 (HBcAg-pre-S) particles, designated as pYA3167, has been constructed as reported in the literature (Schodel, et al., 1996, in Novel strategies in design and production of vaccines. S. Cohen and A. Shafferman, eds., Plenum Press, New York). Accordingly, S. typhi MGN-1191 and MGN-1256 have been transformed with plasmid pYA3167 via electroporation. Immunoblot analysis with HBV pre-S2-specific monoclonal antibody was used to determine the level of expression of the hybrid core pre-S gene in the transformed avirulent S. typhi carrier strains derived from MGN-1191 and MGN-1256. The expression of the hybrid HBcAg-pre-S antigen in .DELTA.phoPQ .DELTA.asd mutant S. typhi strains was determined as follows. Proteins from whole bacterial cell lysates after overnight culture were separated using 12% sodium dodecyl sulfate (SDS-12%), polyacrylamide gel electrophoresis (PAGE) and stained with Coomassie brilliant blue. Results are shown in FIG. 9. Three transformants of MGN-1191 and three transformants of MGN-1256 were studied all of which showed a band at the position of the recombinant antigen (see arrow in FIG. 9). The MGN-1191 transformant #1 (lane 3) was designated .chi.8281 and the MGN-1256 transformant #1 (lane 7) was designated .chi.8280. Both strains express the Vi capsular antigen as determined by positive agglutination with Vi antiserium (Difco).

Detailed Description Text (167):
S. typhi rpoS.sup.+ strains expressing foreign antigens can also be constructed using plasmid vectors with selectable markers other than Asd.sup.+, including genes that confer resistance to drugs such as ampicillin and tetracycline. In addition, the recombinant vector encoding the desired foreign antigen may be constructed using well known techniques such that the vector will insert into the bacterial chromosome by homologous recombination or by transposition.

Detailed Description Text (207):
Bacteriology: Stools and rectal swabs will be inoculated into selenite-cystine broth. Stools must be processed within 48 hours. After overnight incubation at 37.degree. C., subcultures will be made onto XLT-4 agar. Colonies which appear consistent with Salmonella will be processed through API-20 system of identification and confirmation made by a agglutenation with S. typhi O, H, and Vi antisera. These isolates will be saved at -70.degree. C. in 5% glycerol-1% peptone for further analysis (e.g., for the presence of plasmids, for absence or presence of specific DNA sequences using PCR, or for Southern blotting with gene probes for cloned genes).

Detailed Description Text (215):
The following strains and plasmid are on deposit under the terms of the Budapest Treaty, with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. The accession number indicated was assigned after successful viability testing, and the requisite fees were paid. Access to the cultures and plasmid will be available during pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 CFR 1.14 and 35 USC 122. All restriction on availability of the cultures and plasmid to the public will be irrevocably removed upon the granting of a patent based upon the application. Moreover, the designated deposits will be maintained for a period of thirty (30) years from the date of deposit, or for five (5) years after the last request for the deposit, or for the enforceable life of the U.S. patent, whichever is longer.

Should a culture or plasmid become nonviable or be inadvertently destroyed, or, in the case of plasmid-containing strains, lose its plasmid, it will be replaced with a a viable culture. The deposited materials mentioned herein are intended for convenience only, and are not required to practice the present invention in view of the description herein, and in addition, these materials are incorporated herein by reference.

Detailed Description Paragraph Table (1):
TABLE 1
Microorganisms Strain Relevant Designation Genotype Source/Reference/Derivation

Salmonella typhi Strains .chi.3743 ISP1804 Type 46 1983 isolate from Chilean patient, received from D. Hone, Center for Vaccine Development, MD .chi.3744 ISP1820 cys trp ATCC 55116; 1983 isolate from Chilean patient; received from D. Hone .chi.3745 ISP2822 Type E1 ATCC 55114; 1983 isolate from Chilean patient; received from D. Hone .chi.3746 ISP2825 Type E1 1983 isolate from Chilean patient; received from D. Hone .chi.3769 Ty2 Type E1 rpoS cys Louis Baron, Walter Reed Army Institute of Research .chi.3927 Ty2 .DELTA.crp-11 .DELTA.[zhb::Tn10] ATCC 55117 .DELTA.cya-12 .DELTA.[zid-62::Tn10] .chi.4073 Ty2 .DELTA.[crp-cdt]-10 .DELTA.[zhb::Tn10] ATCC 55118 .DELTA.cya-12 .DELTA.[zid-62::Tn10] .chi.8203 cys trp ATCC 9992V; AMC strain Boxhill 58V .chi.8204 cys trp ATCC 33458; CDC 2862-79 .chi.8205 Ty21a galE rpoS cys trp ATCC 33459; CDC 2861-79 .chi.8206 cys trp aroA serC purA155 ATCC 39926; Stanford 531Ty; derivative of CDC10-80 .chi.8207 cys trp ATCC 10749; AMC 42-A-63 .chi.8208 Ty2 cys ATCC 19430; NCTC 8385 .chi.8209 cys trp ATCC 9993; AMC 42-A-63 MGN-1018 Ty2 rpoS cys .DELTA.phoPQ23 Megan Health Inc., St. Louis, MO MGN-1038 ISP1820 cys trp .DELTA.phoPQ23 Megan Health, Inc., St. Louis, MO MGN-1191 ISP1820 cys trp .DELTA.phoPQ23 .DELTA.asdA16 Megan Health, Inc., St. Louis, MO MGN-1256 Ty2 rpoS cys .DELTA.phoPQ23 .DELTA.asdA16 Megan Health, Inc., St. Louis, MO .chi.8280 MGN-1256(pYA3167) .chi.8281 MGN-1191(pYA3167) Salmonella typhimurium Strains .chi.3000 LT2-Z prototroph Received from C. Turnbough .chi.3181 SR-11 pStSR100.sup.+ wild type Isolated by passage from murine Peyer's patch. Gulig and Curtiss, Infect. Immun. 65:2891-2901 (1987). .chi.3339 SL1344 pStSL100.sup.+ hisG rpsL, Animal passaged isolate of SL1344, colicin.sup.+ isolated from liver of moribund mouse after p.o. infection. Gulig and Curtiss, Infect. Immun. 65: 2891-2901 (1987). .chi.3340 SL1344 pStSL100.sup.- hisG rpsL, Virulence plasmid-cured derivative colicin.sup.+ of .chi.3339; Gulig and Curtiss, Infect. Immun. 65:2891-2901 (1987). .chi.3420 SL1344 hisG rpsl xyl fli-8007:: P22HTint (.chi.3376).fwdarw..chi.3339 with selection Tn10 for Tc.sup.R Mot.sup.- Fla.sup.-. .chi.3422 SR-11 fli-8007::Tn10 P22HTint(.chi.3376).fwdarw..chi.3181 with selection for Tc.sup.R Mot.sup.- Fla.sup.- .chi.3679 SR-11 .DELTA.aroA554 P22HTint (.chi.3678).fwdarw. .chi.3181 selecting Tc.sup.r and screening for Aro.sup.- followed by selection for tetracycline sensitivity, Aro.sup.-. .chi.3761 UK-1 wild-type prototroph ATCC 68169; spleenic isolate from infected chick. .chi.4973 SL1344 pStSR100.sup.+ hisG rpsL Nickerson and Curtiss, Infect. rpoS::RR10, colicin.sup.+ Immun., 65:1814-1823 (1997) .chi.8125 SL1344 pStSR100.sup.- hisG rpsL Nickerson and Curtiss, Infect. rpoS::RR10, colicin.sup.+ Immun., 65:1814-1823 (1997) .chi.8214 UK-1 rpoS::RR10 .DELTA.cya-27 .DELTA.crp-27 P22HTint(SF1005).fwdarw.MGN-431 with selection for ampicillin resistance .chi.8215 SR-11 rpoS::RR10 .DELTA.aroA554 P22HTint(SF1005).fwdarw..chi.3679 with selection for ampicillin resistance .chi.8217 UK-1 rpoS::RR10 .DELTA.cya-27 P22HTint(SF1005).fwdarw.MGN-232 with selection for ampicillin resistance MGN-232 UK-1 .DELTA.cya-27 MEGAN Health, Inc.; defined cya deletion derivative of .chi.3761 MGN-431 UK-1 .DELTA.cya-27 .DELTA.crp-27 MEGAN Health, Inc.; defined crp deletion derivative of MGN-232 ATCC 14028s prototroph, Tet.sup.s wild-type invasive strain obtained from F. Heffron SF1005 14028s rpoS::RR10 F. Fang, Univ. Colorado Health Sci. Center E. coli Strains .chi.6212 K-12 F.sup.- .0.80d lacZ .DELTA.M15 This lab .DELTA.(lacZYA-argF) 4169 supE44 .sup.- gyrA recA1 relA1 endA1 .DELTA.asdA4 .DELTA.[zhf-2::Tn10] hsdR17 (r.sub.k -, m.sub.k +) MGN-617 thi-1 thr-1 leuB6 supE44 Megan Health, Inc., St. Louis, MO tonA21 lacY1 recA RP4-2-Tc::Mu pir, .DELTA.asdA4 .DELTA.[zhf-2::Tn10] MGN-758 MGN-617 (pMEG-213) Megan Health, Inc., St. Louis, MO

Detailed Description Paragraph Table (2):
TABLE 2
Phages and Plasmids Description Source/Reference

Bacteriophage P22HTint high frequency generalized Schmeiger, Mol. Gen. Genet. 119:75-88, transducing mutant of the 1972; Jackson et al., J. Mol. Biol. temperate lambdoid phage 154:551-563, 1982; Ray et al., Mol. Gen. P22 Genet. 135:175-184, 1974. P22 H5 clear plaque forming mutant Casjens et al., J. Mol. Biol. 194: of P22HTint 411-422, 1987. Plasmids pSK::rpoS S. typhimurium rpoS gene F. Fang, Univ. Colorado cloned into the EcoRV site Health Sci. Center of pBlueScript/SK pMEG-003 pir-dependent R6K ori Tc.sup.r Megan Health, Inc. asd.sup.+ pMEG-006 pir-dependent R6K ori Tc.sup.r Megan Health, Inc. .DELTA.asdA16 pMEG-068 Contains phoQ gene Megan Health, Inc. pMEG-149 Amp.sup.R mobilizable pir- Megan Health, Inc. dependent suicide vector; containing the sacBR genes from B. subtillis, RK2 mob, R6K ori pYA3433 Contains rpoS gene This lab pMEG-210 phoQ deletion of pMEG-068 Megan Health, Inc. pMEG-213 Derivative of pMEG-149 Megan Health, Inc. containing phoPQ23 defined deletion of pMEG-210 pNEB-193 pUC19 derivative that carries New England Biolabs single restriction sites for unique 8 bp cutters AscI, PacI and PneI within the polylinker region pYA3167 asd - complementing plasmid; Nardelli-Haefliger et al., expresses the Hepatitis B Infect. Immun. 64:5219-5224, 1996 virus (HBV) nucleocapsid pre-S1 and pre-S2 epitopes on HBV core

Detailed Description Paragraph Table (7):
TABLE 7 Ratios of wild-type to rpoS mutants for virulence plasmid-cured S. typhimurium in mouse tissues after peroral coinfection.sup.a Time after Intestinal Intestinal Peyer's Infection Contents Wall.sup.b Patches 3 days 37.7 .+-. 11.8 4.4 .+-. 3.5 UD.sup.c 5 days 3.2 .+-. 1.2 1.2 .+-. 0.3 5.4 .+-. 0.5 .sup.a Approximately equal numbers of .chi.3340 and .chi.8125 (4.0 .times 10.sup.9 CFU and 3.4 .times. 10.sup.9 CFU, respectively) were administere perorally to 10week old BALB/c mice. Mean ratios of CFU/g of tissue for .chi.3344/.chi.8125 .+-. SEM (n = 3) are given. Only bacterial counts greater than 20 CFU/g were considered when calculating the ratios. .sup.b Small and large intestine with Peyer's patches removed. .sup.c Bacterial numbers undetectable at a 1:100 dilution.

Detailed Description Paragraph Table (13):
Deposit Deposit Date ATCC No.
Strains: MGN-1191 November 14, 1997 202054 MGN-1256 November 14, 1997 202053 .chi.8281 November 14, 1997 202056 .chi.8280 November 14, 1997 202055 Plasmid: pYA3433 November 14, 1997 209462

Other Reference Publication (14):
Gulig et al., Plasmid-Associated Virulence of Salmonella typhimurium, Infect. Immun., 55:2891-2901 (1987).

Other Reference Publication (15):
Gulig et al., Cloning and Transposon Insertion Mutagenesis of Virulence GEnes of the the 100-Kilobase Plasmid of Salmonella typhimurium, Infect. Immun. 56:3262-3271 (1987).

Other Reference Publication (16):
Hackett et al., The Colonization of Peyer's Patches by a Strain of Salmonella typhimurium Cured of the Cryptic Plasmid, J. Infect. Dis., 153:1119-1125 (1986).

Other Reference Publication (18):

Kowarz et al., The Salmonella typhimurium katF (rpoS) Gene: Cloning, Nucleotide Sequence, and Regulation of spvR and spvABCD Virulence Plasmid Genes, J. Bacteriol., Bacteriol., 176:6852-6860 (1994).

CLAIMS:

2. The method according to claim 1 wherein the strain of S. typhi comprises an inactivating mutation in a pab gene, a pur gene, an aro gene, asd, a dap gene, nadA, pncB, galE, pmi, fur, rpsL, ompR, htrA, hemA, cdt, cya, crp, phoP, phoQ, rfc, poxR, galU or a combination thereof.

8. The method according to claim 7 wherein the strain of S. typhi comprises an inactivating mutation in a pab gene, a pur gene, an aro gene, asd, a dap gene, nadA, pncB, galE, pmi, fur, rpsL, ompR, htrA, hemA, cdt, cya, crp, phoP, phoQ, rfc, poxR, galU or combination thereof.

12. The method according to claim 11 wherein the strain of S. typhi comprises an inactivating mutation in a pab gene, a pur gene, an aro gene, asd, a dap gene, nadA, pncB, galE, pmi, fur, rpsL, ompR, htrA, hemA, cdt, cya, crp, phoP, phoQ, rfc, poxR, galU or combination thereof.

16. The method according to claim 15 wherein the strain of S. typhi comprises an inactivating mutation in a pab gene, a pur gene, an aro gene, asd, a dap gene, nadA, pncB, galE, pmi, fur, rpsL, ompR, htrA, hemA, cdt, cya, cya, phoP, phoQ, rfc, poxR, galU or combination thereof.

22. The method according to claim 21 wherein the strain of S. typhi comprises an inactivating mutation in a pab gene, a pur gene, an aro gene, asd, a dap gene, nadA, pncB, galE, pmi, fur, rpsL, ompR, htrA, hemA, cdt, cya, crp phoP, phoQ, rfc, poxR, galU or combination thereof.

26. The method according to claim 25 wherein the strain of S. typhi comprises an inactivating mutation in a pab gene, a pur gene, an aro gene, asd, a dap gene, nadA, pncB, galE, pmi, fur, rpsL, ompR, htrA, hemA, cdt, cya, crp, phoP, phoQ, rfc, poxR, galU or combination thereof.

30. The carrier microbe according to claim 29 wherein the avirulent S. typhi comprises an inactivating mutation in a pab gene, a pur gene, an aro gene, asd, a dap gene, nadA, pncB, galE, pmi, fur, rpsL, ompR, htrA, hemA, cdt, cya, crp, phoP, phoQ, rfc, poxR, galU or combination thereof.

35. The immunogenic composition according to claim 34 wherein the strain of S. typhi comprises an inactivating mutation in a pab gene, a pur gene, an aro gene, asd, a dap gene, nadA, pncB, galE, pmi, fur, rpsL, ompR, htrA, hemA, cdt, cya, crp, phoP, phoQ, rfc, poxR, galU or combination thereof.

Terms	Documents

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☐	L3	broth$ same immunogenic$ same antibiot$	2
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DOCUMENT-IDENTIFIER: US 6413751 B1
TITLE: DNA adenine methyltransferases and uses thereof

Brief Summary Text (20):
In another embodiment, this invention provides for antibodies to the methyltransferases encoded by the above-mentioned nucleic acids. Particularly preferred antibodies specifically bind a polypeptide comprising at least 10, more preferably at least 20, 40, 50, and most preferably at least 100, 200, and even 300 contiguous amino acids, or even the full length polypeptide encoded by a nucleic acid selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7; wherein said polypeptide elicits the production of an antiserum or antibody which specifically binds to a polypeptide selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ. ID NO: 6, or SEQ ID NO: 8, wherein the antiserum or antibody preferably does not cross-react with the C. crescentus adenine methyltransferase. The antibody can be polyclonal or monoclonal. The antibody can also be humanized or human.

Brief Summary Text (21):
This invention also provides for cells (e.g., recombinant cells such as hybridomas or triomas) which synthesize any of the above-described antibodies.

Brief Summary Text (22):
This invention also provides for kits for the detection and/or quantification of the above-mentioned nucleic acids. The kit can include a container containing one or more of any of the above identified nucleic acids, amplification primers, and antibodies with or without labels, free, or bound to a solid support as described herein. The kits can also include instructions for the use of one or more of these reagents in any of the assays described herein.

Brief Summary Text (23):
This invention further provides for methods and assays for identification and screening for novel antibiotics that target the methyltransferases of this invention. Such assays include those for screening for inhibitors of DNA methyltransferase activity that comprises: i. contacting in an aqueous reaction mixture a nucleic acid encoding a DNA methyltransferase wherein said methyltransferase has a molecular weight of about 30-45 kilodaltons and binds to a polyclonal antibody that specifically binds to a polypeptide from the group of polypeptides having SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8 with an antisense agent that inhibits the expression of the methyltransferase; and ii. detecting the level of inhibition relative to a control reaction mixture that is substantially identical to the reaction mixture of step i except that the antisense agent is not present in an amount effective to inhibit the expression of the methyltransferase. The methods include both in vivo and in vitro methods. The antisense agents can either be added exogenously or are produced endogenously through conventional recombinant gene methods.

Brief Summary Text (24):
Other methods for screening include methods for assaying for inhibitors of DNA methyltransferase activity comprising the steps of: i. contacting an aqueous reaction mixture containing a DNA methyltransferase wherein said methyltransferase

has a molecular weight of about 30-45 kilodaltons and binds to a polyclonal antibody that specifically binds to a polypeptide from the group of polypeptides having SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8 with an agent that inhibits the biological activity of the methyltransferase; and, ii. detecting the level of inhibition relative to a control reaction mixture that is substantially identical to the reaction mixture of step i except that the inhibitory agent is not present in an amount effective to inhibit the expression of the methyltransferase. The DNA methyltransferase is not contained within a living cell or the assay can be an in vivo assay where the enzyme is inhibited within a living cell.

Brief Summary Text (26):
Finally, this invention also provides therapeutic methods. These include methods of detecting infections with Brucella spp. and H. pylori by detecting the presence or absence of specific sequences of Brucella or H. pylori adenine methyltransferases or by detecting the proteins themselves using antibodies. Other methods include treating conditions caused by Agrobacterium spp., Rhizobium spp, and Helicobacter spp. Other methods involve administering to a mammal a therapeutically effective dose of a composition comprising a methyl transferase inhibitor and a pharmacological excipient. For animal associated bacteria, methods are preferably performed on mammals such as mice, rats, rabbits, sheep, goats, pigs, more preferably on primates including human patients. Of course for plant associated bacteria such as Agrobacterium and Rhizobium spp., the preferred methods are performed on their respective host plants.

Detailed Description Text (28):
The phrase "specifically binds to an antibody" or "specifically immunoreactive with", when referring to a protein or peptide, refers to a binding reaction which is determinative of the presence of the protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein and do not bind in in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, antibodies raised to adenine methyltransferase with the amino acid sequence depicted in SEQ ID NO: 2, 4, 6, or 8 can be selected to obtain antibodies specifically immunoreactive with that adenine methyltransferase and not with other proteins. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

Detailed Description Text (60):
Typically, labelled signal nucleic acids are used to detect hybridization. Complementary nucleic acids or signal nucleic acids may be labelled by any one of several methods typically used to detect the presence of hybridized polynucleotides. The most common method of detection is the use of autoradiography with .sup.3 H, .sup.125 I, .sup.35 S, .sup.14 C, or .sup.32 P-labelled probes or the like. Other labels include ligands which bind to labelled antibodies, fluorophores, chemiluminescent agents, enzymes, and antibodies which can serve as specific binding pair members for a labelled ligand.

Detailed Description Text (62):
The label may also allow indirect detection of the hybridization complex. For example, where the label is a hapten or antigen, the sample can be detected by using antibodies. In these systems, a signal is generated by attaching fluorescent or enzyme molecules to the antibodies or, in some cases, by attachment to a radioactive label. (Tijssen, P., "Practice and Theory of Enzyme Immunoassays," Laboratory Techniques in Biochemistry and Molecular Biology, Burdon, R. H., van

Knippenberg, P. H., Eds., Elsevier (1985), pp. 9-20.)

Detailed Description Text (67):
Methyltransferase may be detected or quantified by a variety of methods. Preferred methods involve the use of specific antibodies.

Detailed Description Text (68):
Methods of producing polyclonal and monoclonal antibodies are known to those of skill in the art. See, e.g., Coligan (1991), Current Protocols in Immunology, Wiley/Greene, NY; and Harlow and Lane (1989), Antibodies: A Laboratory Manual, Cold Spring Harbor Press, NY; Stites et al. (eds.) Basic and Clinical Immunology (4th ed.) Lange Medical Publications, Los Altos, Calif., and references cited therein; Goding (1986), Monoclonal Antibodies: Principles and Practice (2d ed.) Academic Press, New York, NY; and Kohler and Milstein (1975), Nature, 256:495-497. Such techniques include antibody preparation by selection of antibodies from libraries of of recombinant antibodies in phage or similar vectors. See, Huse et al. (1989), Science, 246:1275-1281; and Ward et al. (1989), Nature, 341:544-546. For example, in order to produce antisera for use in an immunoassay, the polypeptide of SEQ ID NO: 2, 4, 6, or 8, or a fragment thereof, is isolated as described herein. For example, recombinant protein is produced in a transformed cell line. An inbred strain of mice or rabbits is immunized with the protein of SEQ ID No. 2, 4, 6, or 8, or a fragment thereof, using a standard adjuvant, such as Freund's adjuvant, and a standard immunization protocol. Alternatively, a synthetic peptide derived from the sequences disclosed herein and conjugated to a carrier protein can be used as an immunogen. Polyclonal sera are collected and titered against the immunogen protein in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Polyclonal antisera with a titer of 10.sup.4 or greater are selected and tested for their cross reactivity against non-adenine methyltransferases or even other adenine methyltransferases, using a competitive binding immunoassay. Specific monoclonal and polyclonal antibodies and antisera will usually bind with a K.sub.D of at least about 0.1 mM, more usually at least about 1 .mu.M, preferably at least about 0.1 .mu.M or better, and most preferably, 0.01 .mu.M or better.

Detailed Description Text (69):
A number of immunogens may be used to produce antibodies specifically reactive with DNA adenine methyltransferases. Recombinant protein is the preferred immunogen for the production of monoclonal or polyclonal antibodies. Naturally occurring protein may also be used either in pure or impure form. Synthetic peptides made using the DNA adenine methyltransferase sequences described herein may also used as an immunogen for the production of antibodies to the protein. Recombinant protein can be expressed in eukaryotic or prokaryotic cells as described above, and purified as generally described above. The product is then injected into an animal capable of producing antibodies. Either monoclonal or polyclonal antibodies may be generated, for subsequent use in immunoassays to measure the protein.

Detailed Description Text (70):
Methods of production of polyclonal antibodies are known to those of skill in the art. In brief, an immunogen, preferably a purified protein, is mixed with an adjuvant and animals are immunized. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the DNA adenine methyltransferase. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the protein can be done if desired. (See Harlow and Lane, supra).

Detailed Description Text (71):
Monoclonal antibodies may be obtained by various techniques familiar to those skilled in the art. Briefly, spleen cells from an animal immunized with a desired

antigen are immortalized, commonly by fusion with a myeloma cell (See, Kohler and Milstein, Eur. J. Immunol. 6:511-519 (1976), incorporated herein by reference). Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods well known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences which encode a monoclonal antibody or a binding fragment thereof by screening a DNA library from human B cells according to the general protocol outlined by Huse, et al. (1989) Science 246:1275-1281.

Detailed Description Text (72):
A particular protein can be measured by a variety of immunoassay methods. For a review of immunological and immunoassay procedures in general, see Basic and Clinical Immunology 7th Edition (D. Stites and A. Terr ed.) 1991. Moreover, the immunoassays of the present invention can be performed in any of several configurations, which are reviewed extensively in Enzyme Immunoassay, E. T. Maggio, ed., CRC Press, Boca Raton, Fla. (1980); "Practice and Theory of Enzyme Immunoassays," P. Tijssen, Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers B. V. Amsterdam (1985); and Harlow and Lane, Antibodies, A Laboratory Manual, supra, each of which is incorporated herein by reference.

Detailed Description Text (74):
In addition, it is possible to produce monospecific antibodies that react to specific DNA methyltransferases from specific species of bacteria as identified herein. Monospecific antibodies are achieved by appropriate cross-absorption with select DNA methyltransferases or by raising antibodies against species specific regions of the amino acid sequence of the transferases. Such unique peptide fragments are routinely identified by sequence comparisons.

Detailed Description Text (76):
Immunoassays in the competitive binding format can be used for the crossreactivity determinations. For example, the protein of SEQ ID NO: 2 can be immobilized to a solid support. Proteins (other methyltransferases, or non-methyltransferases) are added to the assay which compete with the binding of the antisera to the immobilized antigen. The ability of the above proteins to compete with the binding of the antisera to the immobilized protein is compared to the protein of SEQ ID NO: 2. The percent crossreactivity for the above proteins is calculated, using standard calculations. Those antisera with less than 10% crossreactivity with each of the proteins listed above are selected and pooled. The cross-reacting antibodies are optionally removed from the pooled antisera by immunoabsorbtion with the above-listed proteins.

Detailed Description Text (77):
The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay as described above to compare a second protein to the immunogen protein, in this case, the adenine methyltransferase of SEQ ID NO: 2. In order to make this comparison, the two proteins are each assayed at a wide range of concentrations and the amount of each protein required to inhibit 50% of the binding of the antisera to the immobilized protein is determined. If the amount of the second protein required is less than 10 times the amount of the protein of SEQ ID NO: 2 that is required, then the second protein is said to specifically bind to an antibody generated to an immunogen consisting of the protein of SEQ ID NO: 2.

Detailed Description Text (78):
The presence of a desired polypeptide (including peptide, transcript, or enzymatic digestion product) in a sample may be detected and quantified using Western blot analysis. The technique generally comprises separating sample products by gel

electrophoresis on the basis of molecular weight, transferring the separated proteins to a suitable solid support (such as a nitrocellulose filter, a nylon filter, or derivatized nylon filter), and incubating the sample with labeling antibodies that specifically bind to the analyte protein. The labeling antibodies specifically bind to analyte on the solid support. These antibodies are directly labeled, or alternatively are subsequently detected using labeling agents such as antibodies (e.g., labeled sheep anti-mouse antibodies where the antibody to an analyte is a murine antibody) that specifically bind to the labeling antibody.

Detailed Description Text (80):
The polypeptides of this invention may be purified to substantial purity by standard techniques, including selective precipitation with such substances as ammonium sulfate, column chromatography, immunopurification methods, and others. See, for instance, R. Scopes, Protein Purification: Principles and Practice, Springer-Verlag: New York (1982), incorporated herein by reference. For example, the methyltransferase proteins and polypeptides produced by recombinant DNA technology may be purified by a combination of cell lysis (e.g., sonication) and affinity chromatography or immunoprecipitation with a specific antibody to methyltransferase. For fusion products, subsequent digestion of the fusion protein with an appropriate proteolytic enzyme releases the desired polypeptide. The proteins may then be further purified by standard protein chemistry techniques. A specific protocol for purifying the methyltransferases of this invention is provided in Example 1(e).

Detailed Description Text (88):
In still another embodiment, bacterial reporter strains are used to evaluate candidate anti-transferase agents. In such assays, recombinant bacteria are modified to include a reporter gene attached to a nucleic acid encoding the methyltransferase gene. When the genes are expressed, the reporter gene is also expressed and provides a detectable signal indicating the expression of the gene. Anti-methyltransferase agent screens then involve contacting the reporter strains and/or cells, tissues, or organisms prior to or after infection with the reporter strains and subsequently detecting expression levels of the reporter gene.

Detailed Description Text (89):
In addition to screening for antisense agents, this invention provides for methods that facilitate the identification of non-antisense drug candidates especially under conditions of high throughput. The screening for such non-nucleic acid based inhibitory agents commonly involves contacting the target pathogen (e.g. Brucella abortus), and /or a tissue containing the pathogen, and/or an animal, with one or more candidate anti-methyltransferase agents and detecting the presence absence, quantity of the gene product. Alternatively, candidate anti-methyltransferase agents can be identified simply by their ability to bind to the gene or gene product and inhibit its biological activity.

Detailed Description Text (90):
Methods for detecting the biological activity of the methyltransferases are provided herein and include reaction conditions and suitable substrates for methylation. These assays can be used to screen for anti-methyltransferase agents. Absence of the activity of the gene during and/or after contacting of the bacteria, a cell, a tissue, and/or an organism with an anti-transferase agent of interest will indicate that the particular test compound is a likely candidate for an antibiotic.

Detailed Description Text (91):
In view of the foregoing, preferred assays for detection anti-methyltransferase agents fall into the following categories:

Detailed Description Text (103):
Preparation and screening of combinatorial chemical libraries is well known to

those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., U.S. Pat. No. 5,010,175, Furka (1991) Int. J. Pept. Prot. Res., 37: 487-493, Houghton et al. (1991) Nature, 354: 84-88). Peptide synthesis is by no means the only approach envisioned and intended for use with the present invention. Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to: peptoids (PCT Publication No WO 91/19735, Dec. 26, 1991), encoded peptides (PCT Publication WO 93/20242, Oct. 14, 1993), random bio-oligomers (PCT Publication WO 92/00091, Jan. 9, 1992), benzodiazepines (U.S. Pat. No. 5,288,514), diversomers such such as hydantoins, benzodiazepines and dipeptides (Hobbs et al., (1993) Proc. Nat. Acad. Sci. USA 90: 6909-6913), vinylogous polypeptides (Hagihara et al. (1992) J. Amer. Chem. Soc. 114: 6568), nonpeptidal peptidomimetics with a Beta-D-Glucose scaffolding (Hirschmann et al., (1992) J. Amer. Chem. Soc. 114: 9217-9218), analogous organic syntheses of small compound libraries (Chen et al. (1994) J. Amer. Amer. Chem. Soc. 116: 2661), oligocarbamates (Cho, et al., (1993) Science 261:1303), 261:1303), and/or peptidyl phosphonates (Campbell et al., (1994) J. Org. Chem. 59: 658). See, generally, Gordon et al., (1994) J. Med. Chem. 37:1385, nucleic acid libraries, peptide nucleic acid libraries (see, e.g., U.S. Pat. No. 5,539,083) antibody libraries (see, e.g., Vaughn et al. (1996) Nature Biotechnology, 14(3): 309-314), and PCT/US96/10287), carbohydrate libraries (see, e.g., Liang et al. (1996) Science, 274: 1520-1522, and U.S. Pat. No. 5,593,853), and small organic molecule libraries (see, e.g., benzodiazepines, Baum (1993) C&EN, Jan 18, page 33, isoprenoids U.S. Pat. No. 5,569,588, thiazolidinones and metathiazanones U.S. Pat. No. 5,549,974, pyrrolidines U.S. Pat. Nos. 5,525,735 and 5,519,134, morpholino compounds U.S. Pat. No. 5,506,337, benzodiazepines 5,288,514, and the like).

Detailed Description Text (108):
High throughput assays for the presence, absence, or quantification of particular nucleic acids or protein products are well known to those of skill in the art. Similarly, binding assays are similarly well known. Thus, for example, U.S. Pat. No. 5,559,410 discloses high throughput screening methods for proteins, U.S. Pat. No. 5,585,639 discloses high throughput screening methods for nucleic acid binding (i.e., in arrays), while U.S. Pat. Nos. 5,576,220 and 5,541,061 disclose high throughput methods of screening for ligand/antibody binding.

2: Search dam 1979 coli : 6 Select from History

Search PubMed for Search

1: Mutat Res. 1979 Jul;61(2):153-62. Related Articles, Books, LinkOut

Spontaneous mutagenesis in Escherichia coli strains lacking 6-methyladenine residues in their DNA: an altered mutational spectrum in dam- mutants.

Glickman BW.

The mutational spectrum at the lacI locus in a dam-4 strain of Escherichia coli was examined. The observed 20-fold increase in spontaneous mutagenesis in a dam- strain was found to be due to base substitutions, primarily transitions, which had increased 140-fold. Using the trpE997 mutation it was found that the dam mutations also resulted in an increase in frameshift mutagenesis. The mutational spectrum of dam- strains was similar to that found with strains carrying the mutH, mutL, mutS and uvrE mutations thought to result in a defect in the repair of mismatched bases. These results are taken to be consistent with, and to support the hypothesis that, dam- strains are deficient in a post-replicative error-avoidance pathway which allows the directed elimination of mismatch lesions by a mechanism in which parental strands are recognized by their level of DNA methylation.

PMID: 384219 [PubMed - indexed for MEDLINE]

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DATE: Thursday, March 18, 2004

Hide?	Set Name	Query	Hit Count
		DB=USPT; PLUR=YES; OP=AND	
☐	L1	glycerol.clm. same carrier.clm.	280
☐	L2	L1 same pharmaceut$.clm.	49

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 49 of 49 returned.

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1. 6664356. 23 Jan 03; 16 Dec 03. Leach resistant oil based carrier for cosmetically and pharmaceutically active agents. Shih; Jenn S.. 526/328.5; 424/78.22 424/78.24 526/213 526/265 526/303.1 526/307.2 526/307.6 526/317.1 526/319 526/329.2 526/333. C08F220/10.

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2. 6656701. 29 Jan 02; 02 Dec 03. Combination of acid protease enzymes and acidic buffers and uses thereof. Bishop; Michael, et al. 435/23; 424/401 435/24 435/4. C12Q001/37 C12Q001/00 A61K006/00.

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3. 6596266. 20 Feb 01; 22 Jul 03. Compositions containing minoxidil and saw palmetto for treating baldness. Catalfo; Chris, et al. 424/74; 424/70.1 424/725 424/727. A61K007/06 A61K035/78.

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5. 6518311. 25 Jun 01; 11 Feb 03. Use of branched-chain fatty acids and derivatives thereof for the treatment of pain. Kozak; Alexander, et al. 514/613; 554/35 554/79 562/512 564/192. A01N032/18.

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6. 6503946. 15 Aug 01; 07 Jan 03. Preparation for warts. Agholme; Astrid. 514/557;. A61K031/19.

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7. 6503481. 02 May 00; 07 Jan 03. Compositions for aerosolization and inhalation. Thurston; Rachel M., et al. 424/45; 128/200.14 128/200.21 239/690 239/704 239/708 424/1.13 424/1.61 424/1.65 424/43 424/44 514/12 514/2. A61M011/00 A61M015/00 A61K009/12 A61K009/72 B05B005/00.

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8. 6495120. 30 Aug 01; 17 Dec 02. Formulation and system for intra-oral delivery of pharmaceutical agents. McCoy; Randall, et al. 424/45; 424/43 514/2 514/3 514/4. A61K009/12 A61K009/10 A61K038/10 A61K038/28.

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11. 6429197. 30 Apr 99; 06 Aug 02. Metabolic intervention with GLP-1 or its biologically active analogues to improve the function of the ischemic and reperfused brain. Coolidge; Thomas R., et al. 514/21; 424/185.1 514/12 514/2 514/3 530/303 530/308 530/324 530/350. A61K038/00 A61K038/28 C07K005/00.

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☐ 23. 5955624. 07 Jun 95; 21 Sep 99. Growth stimulating factors. Nudelman; Edward, et al. 554/227;. C07C057/00.

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Terms	Documents
L1 same pharmaceut$.clm.	49

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L2: Entry 24 of 49 File: USPT Sep 14, 1999

DOCUMENT-IDENTIFIER: US 5952313 A
TITLE: LPS antagonists and methods of making and using the same

CLAIMS:

5. The composition of claim 1, wherein the pharmaceutically acceptable carrier is saline, mannitol, lactose, starch, magnesium stearate, sodium saccharin, talc, cellulose, glucose, sucrose, magnesium carbonate, water, aqueous dextrose, glycerol, ethanol or a mixture thereof.

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L2: Entry 33 of 49 File: USPT Nov 7, 1995

DOCUMENT-IDENTIFIER: US 5464820 A
TITLE: Specific inhibitors of tissue kallikrein

CLAIMS:

7. The pharmaceutically composition of claim 1 wherein the pharmaceutically acceptable carrier is selected from the group consisting of water, oil, alcohol, saline, glycerol, polysaccharide, starch, and a combination thereof.

First Hit Fwd Refs

L2: Entry 1 of 8 File: USPT Mar 16, 2004

US-PAT-NO: 6706267
DOCUMENT-IDENTIFIER: US 6706267 B1

TITLE: Glucosamine and egg for reducing inflammation

DATE-ISSUED: March 16, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Adalsteinsson; Orn	Kennett Square	PA		
Hunchar; Jeffrey G.	West Chester	PA		
Iyer; Subramanian	Hockessin	DE		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Arkion Life Sciences LLC	Wilmington	DE			02

APPL-NO: 09/ 656712 [PALM]
DATE FILED: September 7, 2000

PARENT-CASE:
RELATED APPLICATIONS This application claims the benefit of U.S. Provisional Application No. 60/153,887, filed Sep. 14, 1999 and U.S. Provisional Application No. 60/192,386, filed Mar. 27, 2000.

INT-CL: [07] A61 K 39/395, C07 K 1/00

US-CL-ISSUED: 424/157.1; 424/130.1, 530/395, 530/300, 530/350, 435/4
US-CL-CURRENT: 424/157.1; 424/130.1, 435/4, 530/300, 530/350, 530/395

FIELD-OF-SEARCH: 424/130.1, 424/157.1, 530/395, 530/300, 530/350, 435/4

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL Clear

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	4357272	November 1982	Polson	260/112R
☐	4367309	January 1983	Kondo et al.	525/54.1
☐	4473551	September 1984	Schinitsky	424/95
☐	4550019	October 1985	Polson	424/85

☐	4748018	May 1988	Stolle et al.	424/87
☐	5772999	June 1998	Greenblatt et al.	424/187.1
☐	6162787	December 2000	Sorgente et al.	514/2
☐	6251863	June 2001	Yue	514/12
☐	6451771	September 2002	Henderson et al.	514/54

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
WO 97/35595	October 1997	WO	
WO 98/04273	February 1998	WO	
WO 99/36077	July 1999	WO	

OTHER PUBLICATIONS

CV-0083A--Specification.

ART-UNIT: 1648

PRIMARY-EXAMINER: Housel; James

ASSISTANT-EXAMINER: Chen; Stacy B.

ATTY-AGENT-FIRM: Krikelis; Basil S.

ABSTRACT:

The invention is directed to a composition and method for the treatment and prevention of inflammation and inflammatory related disorders. The composition is glucosamine in combination with an egg product. It is generally preferred that the egg product is obtained from an avian which has been hyperimmunized with an immungenic mixture and/or which contains an anti-inflammatory composition.

12 Claims, 2 Drawing figures

WEST Search History

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DATE: Thursday, March 18, 2004

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		DB=USPT; PLUR=YES; OP=AND	
☐	L1	coli.clm. same immunogenic.clm. same composition.clm.	15
☐	L2	l1 and safe$	8

END OF SEARCH HISTORY

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L2: Entry 8 of 8 File: USPT May 5, 1998

DOCUMENT-IDENTIFIER: US 5747309 A
TITLE: Bacterial vaccines using vaccine strains of pathogenic bacteria

Brief Summary Text (88):
Stable mutant: a mutant with a low frequency of reversions. In general, mutants with reversion frequencies less than about 10.sup.-7 are considered stable, with reversion frequencies less than 1.times.10.sup.-8 considered safe for vaccine strains.

Other Reference Publication (46):
Kotloff et al., "Safety, Immunogenicity, and Efficacy in Monkeys and Humans of Invasive Escherichia coli K-12 Hybrid Vaccine Candidates Expressing Shigella flexneri 2a Somatic Antigen", Infection and Immunity, vol. 60, No. 6, pp. 2218-2224 (1992).

CLAIMS:

15. A vaccine composition comprising in a pharmaceutically acceptable vehicle at least one E. coli strain, wherein said E. coli strain is an attenuated virulent E. coli strain comprising a fur mutation, said mutation providing attenuation of the virulence of said E. coli strain, wherein said strain retains its immunogenic properties so as to be protectively immunogenic.

42. A vaccine composition comprising in a pharmaceutically acceptable vehicle at least one E. coli strain, wherein said E. coli strain is an attenuated virulent E. coli strain comprising a fur mutation and a pyrimidine pathway mutation, said mutations providing attenuation of the virulence of said E. coli strain, wherein said strain retains its immunogenic properties so as to be protectively immunogenic.

71. A vaccine composition comprising in a pharmaceutically acceptable vehicle at least one E. coli strain, wherein said E. coli strain is an attenuated virulent E. coli strain comprising a pyrimidine pathway mutation, said mutation providing attenuation of the virulence of said E. coli strain, wherein said strain retains its immunogenic properties so as to be protectively immunogenic.